PATENT COOPERATION TREATY

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MERCHANT & GOULD
MINNEAPOLIS, MN 554(

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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To:
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NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	19 SEP 1997
Applicant's or agent's file reference: 600.311WOI1 IPD	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 96/10252	07/06/1996	07/06/1995

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Peter Ehrenreich Telephone No.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
600.311WOI1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 96/ 10252	07/06/1996	07/06/1995
International Patent Classification (IPC) or national classification and IPC		
C12N15/31		
Applicant		
REGENTS OF THE UNIVERSITY OF MINNESOTA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of 6 sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/01/1997	19 SEP 1997
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+ 49-89) 2399-0, Tx: 523656 epmu d Fax: (+ 49-89) 2399-4465	Telephone No.

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1, 2, 4-80, as originally filed,
pages _____, filed with the demand,
pages 3, 3a, filed with the letter of 01/08/97,
pages _____, filed with the letter of _____,

[x] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-23, filed with the letter of 01/08/97,
Nos. _____, filed with the letter of _____,

[x] the drawings, sheets/fig 1/9-9/9, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:
[ ] the description, pages _____.
[ ] the claims, Nos. _____.
[ ] the drawings, sheets/fig _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-15, 18, 19, 22, 23	YES
	Claims 16, 17, 20, 21	NO
Inventive Step (IS)	Claims	YES
	Claims 1-23	NO
Industrial Applicability (IA)	Claims 1-13, 16-21	YES
	Claims 14, 15, 22, 23	NO

2. CITATIONS AND EXPLANATIONS

1) Reference is made to the following document:

D1 : International Immunology, vol. 5, no. 8, pages 869-875

2) The present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1, 9, 12, 13 does not involve an inventive step in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

D1 discloses the preparation of 9 mutants of SPE-A, each with a single amino-acid substitution, and the preparation of a mutant created by deletion of the 10 N-terminal amino acids of SPE-A ("SPEA(-10)"). Several mutations are disclosed to lead to a loss of function. The mutants are expressed as fusion proteins (see "Methods on page 870). D1 also discloses the injection of one of the mutants into mice. It is disclosed that the serum of these mice is capable of specifically inhibiting the

mitogenicity of SPE-A (see page 874, left hand column, second paragraph).

Although novel over D1, the mutations recited in claim 1 cannot be considered to represent a selection which involve an inventive step in that it appears from page 21, lines 16-25 of the present description that they are equivalent at least to mutations made in domain B-beta strands 4 and 5 as disclosed in D1 (see Ala-77, Ala-100 and Ala-104). This also applies to the corresponding nucleotide sequence and host cell of respectively claims 12 and 13.

Given that the mutations disclosed in D1 can also result in the loss of mitogenicity for T-cells (see page 873), the subject-matter of claim 9 also lacks inventiveness.

3) It appears that the single substitutions listed by dependent claims 2-8 do not lead to any new and unexpected effects in comparison to that observed with the single substitutions carried out in D1. Claims 2-8 are therefore not considered to involve an inventive step over D1. The said claims thus do not satisfy the criterions set forth in Article 33(3) PCT.

4) As acknowledged by the Applicant on pages 1-3, the role of SPE-A in toxic shock is well established in the art. The use of mutants equivalent to those disclosed in D1 as a vaccine to reduce symptoms associated with toxic shock is therefore obvious for the skilled person.

Claims 10, 11, 14 and 15 therefore do not satisfy the criterions set forth in Article 33(3) PCT.

5) The mutant SPE-A toxin of Claims 16 and 17 lack novelty over mutant "SPEA(-10)" disclosed D1. The latter is ob-

tained by deletion of the 10 N-terminal amino acids of SPE-A and can thus be considered to have more than one amino acid "changes" when compared to SPE-A. The lack of mitogenic activity of SPEA(-10) on T-cells is also disclosed in D1 (see page 873, left hand column). This also applies to the corresponding nucleotide sequence and host cell of respectively claims 20 and 21.

6) Again, as acknowledged by the Applicant on pages 1-3, the role of SPE-A in toxic shock is well established in the art. The use of mutant "SPE-A(-10)" known from D1 as a vaccine to reduce symptoms associated with toxic shock is therefore obvious for the skilled person. Moreover, such use is explicitely suggested on page 869 (last paragraph) of D1.

Claims 18, 19, 22 and 23 therefore lack an inventive step.

7) The subject-matter of claims 1-13 and 16-21 is susceptible of industrial applicability as defined in Article 33(4) PCT.

8) For the assessment of present claims 14, 15, 22 and 23 on the question as to whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1) The expression "fragment thereof" in claim 1 can be interpreted in so many ways that it renders the scope of the claim unclear, contrary to Article 6 PCT.

2) As regards the function of the claimed mutant, the expression "substantially nonlethal" used in claims 1 and 16 is vague in that it does not specify any precise relation dose/mortality (Article 6 PCT).

staphylococcal toxic shock syndrome toxin 1, staphylococcal enteroxtoxins A, B, Cn, D, E. G and H, and non-group A streptococcal pyrogenic exotoxins. These toxins have similar biochemical properties, biological activities and various degrees of sequence similarity.

The most severe manifestations of STSS are hypotension and shock, that lead to death. It is generally believed that leakage of fluid from the intravascular to the interstitial space is the final cause of hypotension, supported by the observation that fluid replacement therapy is successful in preventing shock in the rabbit model of STSS described above. It has been hypothesized that SPE-A may act in several ways on the host to induce this pathology. Certain single amino acid substitutions in central regions of the SPE-A molecule have been shown to affect the mitogenic activity of and binding to a HLA class II molecules by SPE-A (Hartwig et al. International Immunology 5:5, 869-875 (1993)).

SPE-A has been shown to block liver clearance of endotoxin of endogenous flora's origin, by comprising the activity of liver Kuppfer cells. This appears to cause a significant increase in circulating endotoxin, that through binding to lipopolysaccharide binding protein (LBP) and CD14 signaling leads to macrophage activation with subsequent release of TNF-α and other cytokines. Support for the role of endotoxin in the disease is given by the finding that the lethal effects of SPE-A can be at least partially neutralized by the

administration to animals of polymyxin B or by the use of pathogen free rabbits.

Another modality of induction of shock could be the direct activity of the toxin on capillary endothelial cells. This hypothesis stems from the finding that the staphylococcal pyrogenic toxin TSST-1 binds directly to the human umbilical cord vein cells and is cytotoxic to isolated porcine aortic endothelial cells.

WHAT IS CLAIMED IS:

1. A mutant SPE-A toxin or fragment thereof, the mutant SPE-A toxin comprising one to six amino acid substitutions and being substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin;

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

2. The mutant SPE-A toxin of claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

3. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic acid.

4. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

5. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

6. The mutant SPE-A toxin of claim 5, wherein the substitution is asparagine-20 to aspartic acid.

7. The mutant SPE-A toxin of claim 5, further comprising substitution of cysteine-98, or lysine-157.

8. The mutant SPE-A toxin of claim 7, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

9. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A toxin.

10. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at leaast one mutant SPE-A toxin according to claim 1.

11. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

12. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

13. A stably transformed host cell comprising a DNA sequence according to claim 12.

14. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 10 to an animal.

15. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 10 to an animal.

16. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least two amino acid changes and is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

17. The mutant SPE-A toxin of claim 16, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains

mitogenicity comparable to that of the wild type SPE-A toxin.

18. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 16.

19. A pharmaceutical composition comprising: a mutant SPE-A according to claim 16 in admixture with a physiologically acceptable carrier.

20. A DNA sequence encoding a mutant SPE-A toxin according to claim 16.

21. A stably transformed host cell comprising a DNA sequence according to claim 29.

22. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 18 to an animal.

23. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 18 to an animal.

UNITED STATES DEPARTMENT OF COMMERCE
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OCTOBER 31, 1996

PTAS

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UNITED STATES PATENT AND TRADEMARK OFFICE
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DOC DATE: 06/28/1996

ASSIGNOR:
ROGGIANI, MANUELA

DOC DATE: 06/28/1996

ASSIGNOR:
STOEHR, JENNIFER

DOC DATE: 06/28/1996

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DOC DATE: 06/28/1996

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SERIAL NUMBER:
PATENT NUMBER:
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FILING DATE:
ISSUE DATE:

JACQUELINE MOORE, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

Rec'd 8-21-96

08-23-1996

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FORM PTO-1595
(Rev. 6-93)
OMB No. 0651-0011 (exp. 4/94)
M&G-600.311WO11

100259442

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

AUG 21 1996

To the Commissioner of Patents and

1. Name of conveying party(ies):

Patrick M. Schlievert
Manuela Roggiani
Jennifer Stoehr
Douglas Ohlendorf

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies):

REGENTS OF THE UNIVERSITY OF MINNESOTA
Morrill Hall
100 Church Street, South East
Minneapolis, Minnesota 55455

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of conveyance:

☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other:

Execution Date: June 28, 1996

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: _____

A. Patent Application No.(s)

PCT/US96/10252

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Alan W. Kowalchyk
Address: Merchant, Gould, Smith, Edell, Welter & Schmidt
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402-4131

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41): $40.00
☒ Enclosed
☐ Authorized to be charged to deposit account

8. Please charge any additional fees or credit any overpayments to our Deposit account number: 13-2725

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To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Alan W. Kowalchyk
Name of Person Signing

Alan W. Kowalchyk
Signature

20 August 1996
Date

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060 JS 08/23/96 SCHLIEVERT P 3 581 40.00 CK

ASSIGNMENT

WHEREAS, WE, Patrick M. Schlievert residing at 5305 Birchcrest Drive, Edina, MN 55436; Manuela Roggiani residing at 100 2nd Street SE, #803, Minneapolis, MN 55414; Jennifer Stoehr residing at 3981 Woodridge Circle, Vadnais Heights, MN 55127; and Doulgas Ohlendorf residing at 9397 Olympia Drive, Eden Prairie, MN 55347, repsectively, made certain new and useful inventions and improvements as described in the international patent application Serial No. PCT/US96/10252 which is entitled MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE with an international filing date of June 7, 1996.

AND WHEREAS, Regents of the University of Minnesota, a corporation organized and existing under and by virtue of the laws of the State of Minnesota, and having an office and place of business at Morrill Hall, 100 Church Street, South East, Minneapolis, Minnesota 55455 (hereinafter "Assignee") is desirous of acquiring the entire right, title and interest in and to said inventions, improvements and application and in and to the Letters Patent to be obtained therefor;

NOW THEREFORE, to all whom it may concern, be it known that for and in consideration of the sum of One Dollar and other good and valuable considerations, the receipt and sufficiency whereof is hereby acknowledged, I/we have sold, assigned, and transferred, and by these presents do sell, assign and transfer unto said Assignee, its successors or assigns, the entire right, title and interest for all countries in and to all inventions and improvements disclosed in the aforesaid application, and in and to the application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of foreign countries which may be granted therefrom; and I/we do hereby authorize and request the Commissioner of Patents and Trademarks to issue any and all United States Letters Patent for the aforesaid inventions and improvements to the Assignee as the assignee of the entire right, title and interest in and to the same, for the use of the Assignee, its successors and assigns.

AND, for the consideration aforesaid, I/we do hereby agree that I/we and my/our executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Assignee, its successors and representatives all facts known to me/us relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in said Assignee, its successors or assigns the entire right, title and interest in and to the improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

AND, furthermore I/we covenant and agree with said Assignee, its successors and assigns, that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by me/us and that full right to convey the same as herein expressed is possessed by me/us.

IN TESTIMONY WHEREOF. I have hereunto set my hand this 28 day of June, 1996.

Patrick M. Schlievert

STATE OF Minnesota)
) ss.
COUNTY OF Hennepin)

On this 28th day of June, 1996 before me personally appeared Patrick M. Schlievert to me known and known to me to be the person described in and who executed the foregoing instrument, and he/she duly acknowledged to me that he/she executed the same for the uses and purposes therein set forth.

[SEAL] LINDA MCGEARY
NOTARY PUBLIC - MINNESOTA
HENNEPIN COUNTY
My Commission Expires
Jan. 31, 2000

Notary Public

IN TESTIMONY WHEREOF, I have hereunto set my hand this 28 day of June, 1996.

Manuela Roggiana

STATE OF Minnesota)
) ss.
COUNTY OF Hennepin)

On this 28th day of June, 1996 before me personally appeared Manuela Roggiana to me known and known to me to be the person described in and who executed the foregoing instrument, and he/she duly acknowledged to me that he/she executed the same for the uses and purposes therein set forth.

[SEAL] LINDA MCGEARY
NOTARY PUBLIC - MINNESOTA
HENNEPIN COUNTY
My Commission Expires
Jan. 31, 2000

Notary Public

IN TESTIMONY WHEREOF, I have hereunto set my hand this 28 day of June, 1996.

Jennifer A. Stoehr
Jennifer Stoehr

STATE OF Minnesota)
) ss.
COUNTY OF Hennepin)

On this 28 day of June, 1996 before me personally appeared Jennifer Stoehr to me known and known to me to be the person described in and who executed the foregoing instrument, and he/she duly acknowledged to me that he/she executed the same for the uses and purposes therein set forth.

LINDA MCGEARY
NOTARY PUBLIC - MINNESOTA
HENNEPIN COUNTY
My Commission Expires
Jan. 31, 2000

Linda McGeary
Notary Public

IN TESTIMONY WHEREOF, I have hereunto set my hand this 27 day of June, 1996.

Douglas Ohlendorf

STATE OF Minnesota)
) ss.
COUNTY OF Hennepin)

On this 28 day of June, 1996 before me personally appeared Douglas Ohlendorf to me known and known to me to be the person described in and who executed the foregoing instrument, and he/she duly acknowledged to me that he/she executed the same for the uses and purposes therein set forth.

LINDA MCGEARY
NOTARY PUBLIC - MINNESOTA
HENNEPIN COUNTY
My Commission Expires
Jan. 31, 2000

Linda McGeary
Notary Public

Rev: January 2, 1996